Journal of Medical Virology 51:189–197 (1997)

Relative Functional Affinity of Specific Anti-Core IgG in Different Categories of Hepatitis B Virus Infection

H.I.J. Thomas*

Department of Virology, Preston Public Health Laboratory, Royal Preston Hospital, Preston, United Kingdom

While resolution of hepatitis B virus (HBV) infection occurs in most cases, a carrier state can exist in which the HBV surface antigen (HBsAg) persists. Some carriers are also positive for the HBV ''e'' antigen (HBeAg), indicative of high viral replication. Others are HBV ''e'' antibody (anti-HBe)-positive carriers in whom there appears to be a fall in the level of viral replication with the appearance of antibodies against the ''e'' antigen. The former group of carriers is considered to be at a higher risk of transmitting HBV infection than the latter. In order that a carrier state may occur, some degree of tolerance to the infectious agent must exist. A study of the rate of increase of specific antibody avidity following infection provides a means of assessing the maturity of the immune response to an infectious agent. Since antibodies specific for the HBV core antigen (HBcAg) are produced in almost all cases of HBV infection and the HBeAg and HBcAg share a large number of amino acids and some B- and T-cell epitopes, the increase in the avidity of antibodies against the HBV core antigen (anti-HBc) in cases of acute, resolving HBV infection and in HBV carriers has, therefore, been studied. An increase in the avidity of specific antibody, similar to that seen in other viral infections, was observed following acute, resolving infection. However, low avidity antibody persisted longer in carriers who remained positive for HBeAg, whereas in cases where there were antibodies specific for HBeAg, the anti-HBc antibody was of high avidity. Analysis of sequential sera from carriers who seroconverted from HBeAg-positive to anti-HBe-positive showed that an increase in anti-core avidity could predate seroconversion from HBeAg-positive to anti-HBe-positive status. Thus, anti-HBc avidity studies may be of diagnostic and prognostic significance. ***J. Med. Virol. 51:189–197, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: HBV; low avidity antibody; acute infection; chronic infection; anti-HBc

INTRODUCTION

The interaction between the hepatitis B virus (HBV) and the infected patient is a complex one [Eddleston, 1993], and the immune response to the virus can be either beneficial or damaging to the patient. Most cases of HBV infection resolve over a period of a few months. HBV surface antigen (HBsAg) is generally detectable prior to the onset of symptoms but not beyond the acute stage except in chronically infected carriers. Shortly after the appearance of HBsAg, the soluble component of the core "e" antigen, HBeAg, is detectable in the serum and is indicative of a high degree of viral replication.

The core antigen (HBcAg) itself is highly immunogenic and gives rise to vigorous T- and B-cell responses [Milich and McLachlan, 1986; Ferreri et al., 1986]. Nonneutralising antibodies against the core antigen (anti-HBc) then appear (anti-HBc IgM first, then anti-HBc IgG). A few weeks later, in acute, resolving cases and in some carriers, HBeAg disappears and is replaced by anti-HBe antibodies (anti-HBe). Despite the presence of these antibodies, antigen production may continue. However, the appearance of anti-HBe antibodies usually signifies a reduction in viral replication and resolution of an acute case or the appearance of a milder, chronic infection with low or undetectable virus levels. The appearance of anti-HBsAg antibodies (anti-HBs) later still generally implies resolution of the infection.

HBV infection can lead to a persistent carrier state in about 5–10% of cases. These cases remain HBsAg-positive. They are also positive for HBeAg for varying periods, with minimal damage to the liver. The persistence of HBeAg is indicative of a high level of viral replication in the liver [Blum, 1993], and, whilst they remain positive for HBeAg, these cases, usually anti-HBe-negative by commercial kits, are considered to be highly infectious. There is some evidence to suggest

*Correspondence to: H.I.J. Thomas, Department of Virology, Preston Public Health Laboratory, Royal Preston Hospital, PO Box 202, Sharoe Green Lane, Preston PR2 4HG, U.K.

Accepted 24 September 1996

that these cases are not truly anti-HBe-negative when more sensitive tests are employed [Maruyama et al., 1993]. During the later stages of infection, these cases may develop chronic hepatitis. It is believed that the immune response to virus-infected hepatocytes is responsible for the hepatitis [Carman et al., 1993; Carman and Thomas, 1993], although it is not clear whether it is due to a cellular or a humoral response or both.

Eventually, these cases may become HBeAg-negative and seroconvert to anti-HBe-positive, detectable by commercial kits used routinely. About the same time as anti-HBe appears, mutant (precore variants) viruses appear which cannot produce HBeAg [Carman et al., 1989]. During this period of seroconversion, their hepatitis becomes more severe.

A satisfactory explanation of why some cases of HBV infection resolve whilst others proceed to a carrier state does not exist at present. Nor is there an explanation of why HBeAg-positive carriers seroconvert to anti-HBe-positive after some (sometimes considerable) time. This study was undertaken in order to see if the carrier status in patients could be explained by an inadequate or immature immune response to the viral core antigen.

The avidity, or functional affinity, of an antibody is a measure of the combined strength of the bonds between the antibody Fab fragment and its specific epitope. Early in an infection, or antigenic challenge, the avidity of the majority of the antibodies produced is low [Eisen and Siskind, 1964; Webster, 1968] and the formation of antigen-antibody complexes slower [Foote and Milstein, 1991] than later in the immune response. The maturation of the immune response to protein antigens, in terms of isotype switch and an increase in antibody avidity with time after infection, is dependent on a complex interrelationship between T cells, B cells, antigen-presenting cells (APC), and cytokines [Austyn, 1989; Gershon and Paul, 1971; Steward and Steensgard, 1983; Liu et al., 1992; Rizzo et al., 1992; Nossal, 1993; Berek and Ziegner, 1993]. The absence of any one or more of these components of the immune system will lead to at least an alteration in the rate of avidity maturation, as can be seen in the response to infection in the developing foetus [Thomas et al., 1993].

The reaction between the antigen and low avidity antibody *in vitro* can be prevented easily [Inouye et al., 1984; Devey et al., 1988; Thomas and Morgan-Capner, 1988, 1991], or the bonds can be broken after formation [Hedman and Rousseau, 1989; Thomas and Morgan-Capner, 1991] by chaotropic agents or protein denaturants. As time progresses, however, the avidity increases (avidity maturation) as both hypermutation of the V region of the immunoglobulin gene in the B cells occurs and declining antigen concentrations favour selection of high avidity, antibody-secreting B-cell clones [Steward, 1981]. The avidity of the specific antibody produced can influence markedly the biological properties of sera [Steward and Steengard, 1983]. For example, high avidity specific antibody has been shown to be more effective in neutralising foot and mouth disease virus than low avidity specific antibody [Steward et al., 1991]. Evidence also exists to suggest that low avidity specific antibody is associated with a decreased immune elimination of antigen and the formation and persistence of immune complexes within tissues with ensuing tissue damage [Steward, 1979; Devey and Steward, 1980]. That there appears to be some abnormality in immune maturation in cases of chronic HBV infection is already evident from the work of Careoda et al. [1982] and Ljunggren et al. [1991]. Both groups showed that HBsAg/IgM complexes were cleared rapidly from resolving cases of acute HBV infection but persisted in HBeAg-positive carriers. The seroconversion from HBeAg-positive status to anti-HBe antibody status appeared to coincide with the disappearance of HBsAg/IgM complexes. Brown et al. [1984] showed that the avidity of anti-HBsAg antibodies was higher in recovered (resolved) cases of HBV infection than in cases of chronic liver disease (hepatocellular carcinoma, alcoholic liver disease, and alcoholic cirrhosis) who were anti-HBs-positive as a result of a previous HBV infection. Wen et al. [1990] showed that the avidity of anti-core antibodies in HBsAg-positive asymptomatic carriers (neither their "e" status nor their HBV-DNA status was defined) was greater than in patients with chronic liver disease and suggested that the larger quantities and lower avidity of the anti-core antibodies in the latter cases played a role in the pathology of the disease.

In this study, the avidity of anti-core antibodies in the sera of two types of HBsAg carrier (HBeAg-positive and anti-HBe antibody-positive) and in sequential sera from carriers who seroconverted from HBeAg-positive to anti-HBe-positive has been compared with the avidity of anti-core antibodies in sera from cases of acute, resolving HBV infection.

MATERIALS AND METHODS

Sera

Remote, resolved infection cases. Fifty-one sera from 46 cases of remote, resolved HBV infection, without HBsAg for more than 2 years after acute infection, were examined.

Acute cases. One hundred twenty-nine sera were taken from 46 cases of acute, resolving infection; of these, 88 were HBsAg-positive, one was HBsAg +/– and 40 were HBsAg-negative.

"e" Antigen-positive, HBsAg-positive carriers. Seventy-one sera from 26 HBeAg positive carriers were examined; of these, 28 were anti-HBc IgM-positive, two were equivocal, 39 were anti-HBc IgM-negative, and the anti-HBc IgM status of two sera was not known.

Anti-HBe antibody-positive, HBsAg-positive carriers. Sixty-three sera from 41 anti-HBe antibody-positive carriers were examined.

HBsAg-positive, "e" antigen to "e" antibody seroconverters. Forty-one sera from 23 cases were examined; of these, 10 sera (from seven cases) were "e" antigen-positive, 24 (from 16 cases) were anti-"e" antibody-positive, and seven were negative for both "e" antigen

and anti-"e" antibody (i.e., in the process of seroconverting).

The HBeAg-positive sera (i.e., pre-"e" seroconversion sera) from 16 cases were not available for avidity studies. However, these cases were included in this study because their progress over the years had been carefully monitored in this laboratory and their "e" seroconversion noted.

Patients were classed as carriers on the basis of past clinical history, HBV serology, and the persistence of HBsAg for longer than 6 months. Serology for "e" status was carried out over a period of years, using the most reliable commercial kits available at the time. Consistency was observed in so far as sera from cases which appeared to be HBeAg or anti-HBe antibody-positive remained so over a considerable period of time. Those cases which seroconverted from HBeAg-positive to anti-HBe antibody-positive did so over a period of time and remained HBeAg-negative, anti-HBe antibody-positive after seroconversion.

Methods

The assessment of the relative avidity (functional affinity) of the anti-core IgG was carried out using adaptations of two previously published methods. Both enzyme-linked immunosorbent assay (ELISA) methods were developed to differentiate between primary rubella and rubella reinfection [Thomas and Morgan-Capner, 1988, 1991; Hedman and Rousseau, 1989] and extended to differentiate between varicella and zoster infections [Thomas et al., 1990] and to recent and long-term HIV infection [Thomas et al., 1996].

In both methods, the wells of Nunc Polysorp microtitre plates were coated with 50 μl of an optimum concentration (10 μg/ml) of recombinant, baculovirus-derived HBcAg (prepared using the HBV core capsid gene from behind the polyhedron gene promotor) in carbonate/bicarbonate coating buffer, pH 9.6. The antigen was a very generous gift from Dr E. Gould and colleagues at the Institute of Virology, Oxford, UK. The optimum concentration was determined using sera from HBV infections which had resolved over 2 years previously (remote sera likely to contain high avidity anti-HBc antibody) and sera from current, acute HBV cases (acute sera likely to contain low avidity antibody).

In the diethylamine (DEA) shift (DSV) method [Thomas and Morgan-Capner, 1988], serum dilutions were prepared, in parallel, in antigen-coated wells of Polysorp microtitre plates (Nunc, Gibco BRL, Life Technology Ltd., Paisley, UK), in serum diluent (5% normal goat serum [Sera-Lab Ltd., Crawley Down, W. Sussex, UK] in phosphate-buffered saline + Tween 20 [5% NGS/PBST]) alone and in serum diluent containing the protein denaturant DEA (BDH, Poole, UK). After incubation at room temperature for 1 hr, wells were washed six times with PBST and any specific antibody remaining attached to the wells was detected by affinity-isolated goat anti-human IgG gamma chain peroxidase conjugate (Tago, Inc., Burlingame, CA, obtained from TCS Biologicals, Botolph Claydon, UK) and orthophenylenediamine/H_2O_2 (OPD) substrate. Curves of optical densities (ODs), read at dual wavelengths 492 and 620 nm against the reciprocal of the $\log_{10}$ of the serum dilutions, were drawn for each diluent and the distance apart, at half the maximum OD, measured to give the DEA shift value (DSV). Low avidity specific antibody is prevented from attaching to the solid phase-bound antigen more easily than high avidity specific antibody. Thus, the DSV is larger with sera from cases with low avidity specific antibody as the DEA-treated serum curve is shifted further to the left. A minimum of six sera from randomly chosen, resolved cases (remote sera) of HBV infection (HBsAg not detectable for >24 months) and at least one serum from a recent, acute case of HBV infection were included in every experiment. The mean (x) of the DSVs of the remote sera was calculated and added to 3 and 5 standard deviations (SD). A test serum was classed as positive for low avidity specific anti-HBc IgG if its DSV was $>x + 5$ SD (DSV+), equivocal if $\geq x + 3$ SD but $\leq x + 5$ SD (DSV+/−), and negative if $<x + 3$ SD (DSV−).

An index (DSV index) [Thomas et al., 1996] of the avidity of the anti-core antibody was also determined by the calculation:

$$\text{DSV index} = \frac{(\text{DSV of test serum} - \times \text{ of remote serum controls})}{1 \text{ SD of remote serum controls}}$$

A high value for the DSV index indicated the presence of a high proportion of low avidity anti-HBc antibody in a serum.

The avidity index (AI) method, originally developed by Hedman and Rousseau [1989] also for rubella serology, was adapted [Thomas and Morgan-Capner, 1991]. Fifty microlitres of a single dilution of serum (1 in 100) was applied to each of four antigen-coated wells and allowed to incubate for 1 hr at room temperature. Two wells were soaked three times, each time for 5 min, in PBST wash buffer and two were similarly soaked in 5% NGS/PBST containing DEA. The wells were then washed six times with PBST and drained before completing the ELISA as above. An AI for each serum was calculated as follows:

$$\text{AI} = \frac{\text{average OD of wells soaked with 50 mM DEA}}{\text{average OD of wells soaked with PBST}} \times 100$$

Once again, a minimum of six sera from cases of remote HBV infection and a minimum of one serum from a recent acute case were included in every experiment. Sera were positive (AI+) for low avidity anti-core antibody if they had an AI <30%, equivocal (AI+/−) if the AI was ≥30% but ≤50%, and negative (AI−) if >50%.

RESULTS

Remote Infection

One of the 51 (1.96%) sera from cases of remote infection was positive by the DSV method (DSV = 0.65;

TABLE I. HBc-Specific IgG Avidity Results in Relation to HBsAg Status in Sera From Cases of Acute, Resolving HBV Infection

Anti-HBc IgG avidity	HBsAg + (n = 88)	HBsAg +/– (n = 1)	HBsAg – (n = 40)
DSV +	74 (84)	1	21 (52.5)
DSV +/–	4 (4.5)		5 (12.5)
DSV –	2 (2.3)		14 (35)
[Ab] too low[a]	8 (9.1)		
AI +	76 (86.4)	1	10 (25)
AI +/–	3 (3.4)		11 (27.5)
AI –	9 (10.2)		18 (45)
Serum not tested			1

DSV, diethylamine shift value test results; AI, avidity index result. + denotes the presence of low avidity specific anti-HBc IgG; +/– denotes an equivocal result; – denotes that the avidity of the specific anti-HBc IgG was indistinguishable from that seen in sera from cases of infection in the distant past (remote cases).
n, number of sera in the group. Numbers in parentheses are percentages.
[a][Ab] too low indicates that there was insufficient anti-HBc IgG in the serum to allow suitable dilution curves to be drawn for the assessment of avidity by the DSV method.

x + 5 SD = 0.64) and two (3.9%) gave equivocal results for low avidity specific anti-HBc IgG. Three sera (5.9%) were positive and three (5.9%) gave equivocal results in the AI method. One of the sera positive by the AI method was also equivocal for low avidity anti-HBc IgG by the DSV method.

Acute HBV Infection

Table I shows the relative avidity of anti-HBc IgG in sera from cases of acute, resolving HBV infection. The results are broken down into HBsAg categories. The majority (⩾84%) of sera positive for HBsAg contained low avidity anti-HBc IgG. Just over half (52.5%) of the sera negative for HBsAg also contained low avidity anti-HBc IgG by the DSV method; 25% were shown to contain low avidity antibody by the less sensitive AI method. The largest percentage of sera negative for low avidity anti-HBc IgG were also negative for HBsAg.

All sera from this group which were HBsAg- and HBeAg-positive (n = 35) contained low avidity anti-HBc IgG by both methods. Of those sera known to be HBsAg- and anti-HBe antibody-positive (n = 24), 21 (88%) contained low avidity anti-HBc IgG by the DSV method and 19 (79%) contained low avidity specific antibody by the AI method. Of the HBsAg-negative sera known to be anti-HBe antibody-positive (n = 21), 14 (67%) contained low avidity anti-HBc IgG by the DSV method but only four (24%) did so by the AI method.

Table II compares the presence of low avidity anti-HBc IgG with the presence of HBeAg in cases of acute, recent HBV infection. The majority of sera contained low avidity anti-HBc irrespective of the HBeAg status. However, the high avidity anti-HBc antibodies (DSV–, AI–) were detected in anti-HBe IgG-positive sera and in sera where the HBeAg status was not known. These latter sera were taken from cases at more advanced stages of infection (100% were HBsAg-negative) and likely to contain anti-HBe antibodies.

Figure 1 shows the decrease in the amount of low avidity anti-HBc IgG (the DSV index) in sera from cases of acute HBV infection with time after the onset of symptoms. Anti-HBc antibody avidity index results in sequential sera from individual patients are shown by lines. The relationship between anti-HBc antibody avidity and HBsAg status is also shown.

HBsAg-Positive, HBeAg-Positive Carriers

Overall, 36 of the 71 sera in this group (50.7%) contained low avidity anti-HBc IgG by the DSV method, 14 (19.7%) gave equivocal results, and 21 (29.6%) were negative. Of the 36 sera which contained low avidity anti-core IgG, 16 (44.4%) were known to be anti-HBc IgM-positive. Eight (38.1%) of the 21 sera which did not contain low avidity anti-core IgG (i.e., contained high avidity anti-core antibody) were HBc-specific IgM-positive, as were the four of the 14 (28.6%) sera equivocal for low avidity specific antibody by the DSV method. The anti-HBc IgM status of two sera was unknown and insufficient serum remained to test.

Only three of this group of sera (4.2%) contained low avidity anti-core IgG by the AI method, and two of those were also anti-HBc IgM-positive. Four sera gave equivocal results; one was anti-HBc IgM-positive and three were anti-HBc IgM-negative. The remaining 62 (87.3%) sera were negative for low avidity by the AI method. Two sera (anti-HBc IgM-negative) were not assessed by the AI method.

HBsAg-positive, Anti-HBe Antibody-Positive Carriers

None of this group of sera contained low avidity anti-core IgG by either method, although two (3.2%) sera gave equivocal results by the DSV method and one (1.6%) gave an equivocal result by the AI method. Both were anti-core IgM-negative.

HBsAg-Positive, HBeAg Positive to Anti-HBe Antibody-Positive Seroconverters

HBeAg-positive. One (10%) of the 10 sera in this group (Table III) contained low avidity anti-core IgG by the DSV method (anti-HBc IgM status not known). It was taken 4 years after the first "e" antigen-positive specimen was received in this laboratory. None of the sera in this group was positive by the AI method. Of the nine (90%) sera negative by the DSV method, two were borderline for anti-HBc IgM, four were negative, and the anti-HBc IgM status of the other three sera was not known. Of the 10 sera negative by the AI method, two were equivocal for anti-HBc IgM, four were negative, and the others were of unknown anti-HBc IgM status.

"e" Antigen, "e" antibody-negative (i.e., in the process of seroconversion). None of the seven sera in this group contained low avidity anti-core IgG (i.e., had high avidity anti-HBc antibody) by either avidity method. They were also known to be negative for anti-HBc IgM.

"e" Antibody-positive. None of the 24 sera in this group contained low avidity anti-HBc IgG by either method, but two (8%) were equivocal for low avidity

TABLE II. HBc-Specific IgG Avidity Results in Relation to HBeAg Status in Sera From Cases of Acute, Resolving HBV Infection

	HBeAg + (n = 41)	anti-HBe + (n = 38)	HBeAg+/– (n = 1)	HBeAg – and anti-HBe – (n = 1)	HBeAg status unknown (n = 48)
HBsAg+	35	24			
HBsAg–	6	14			
Anti-HBc IgG avidity					
DSV +	40 (97.6)	30 (78.9)	1 (100)	1 (100)	30 (62.5)
DSV +/–	1 (2.4)	2 (5.3)	0	0	5 (10.4)
DSV –	0	6 (15.8)	0	0	13 (27.1)
AI +	36 (87.8)	21 (55.3)	1 (100)	1 (100)	27 (56.3)
AI +/–	0	7 (18.4)	0	0	6 (12.5)
AI –	5 (12.2)	10 (26.3)	0	0	15 (31.3)

DSV, diethylamine shift value test results, AI, avidity index result.
+ denotes the presence of low avidity specific anti-HBc IgG; +/– denotes and equivocal result; – denotes that the avidity of the specific anti-HBc IgG was indistinguishable from that seen in sera from cases of infection in the distant past (remote cases). n, number of sera in the group. Numbers in parentheses are percentages.

anti-HBc IgG by the DSV method. Both were negative for anti-HBc IgM. None of the "e" antibody-positive sera in this group contained low avidity anti-HBc IgG by the AI method.

Timing of appearance of high avidity anti-HBc IgG in relation to "e" antigen/"e" antibody status in seroconverters. Table IV shows that the avidity of anti-core antibody was always high prior to loss of detectable "e" antigen except in one case (case G.C.). However, the avidity of the anti-HBc IgG in this "e" antigen-positive serum was high by the less sensitive of the two methods used, suggesting that the antibody response was beginning to mature.

DISCUSSION

Antibodies produced early in the immune response are of low functional affinity (avidity) and can easily be prevented from combining with their specific antigen [Inouye et al., 1984; Devey et al., 1988; Thomas and Morgan-Capner, 1988; Thomas et al., 1991, 1992] or broken apart from an antibody-antigen complex [Hedman and Rousseau, 1989; Thomas and Morgan-Capner, 1991]. Infection with viruses such as rubella result in the production of specific IgG antibodies of increasing functional affinity (avidity) with time after infection [Thomas et al., 1992]. Even infection with a virus which later becomes latent in the host, such as varicella zoster virus (VZV), leads to the production of high avidity specific IgG antibodies [Thomas et al., 1990; Kangro et al., 1991].

In cases of recent infection with the human immunodeficiency virus (HIV), the avidity of antibodies produced against the transmembrane protein gp41 increased over several months after seroconversion [Radkowski et al., 1993; Thomas et al., 1996], but the avidity of antibodies specific for the core protein p24 did not mature in all patients [McRae et al., 1991; Thomas et al., 1996]. It was also observed that maturation of the anti-gp41 response could occur in the face of nonmaturation of the anti-p24 and anti-p17 responses [Thomas et al., 1996], showing that individual antigens of an infectious agent can give rise to different responses within the same individual.

Avidity studies have shown that anti-HBs in resolved cases of HBV infection was of higher avidity than in patients with chronic liver disease [Brown et al., 1984]. However, not all patients produce anti-HBs antibodies, and those who do usually produce them after seroconversion from HBeAg-positive to anti-HBe-positive. This study investigated the avidity of anti-HBc antibodies produced prior to the HBe seroconversion event. Studies on anti-HBc antibodies, already carried out, have revealed that anti-HBc antibodies were of higher avidity in asymptomatic carriers than in symptomatic carriers [Wen et al., 1990], but the authors did not relate their avidity findings to the "e" status or the HBV-DNA status of their patients.

The highly immunogenic HBcAg gives rise to high titres of anti-HBc, which are of the IgG isotype generally unless there has been a reactivation (carriers), when anti-HBc IgM antibodies may reappear. Both anti-HBc isotypes may remain present in declining titres over many years [Hoofnagle et al., 1973]. HBeAg is a pre-core product and is structurally related to HBcAg [Carman et al., 1993]. Whilst HBcAg is an essential component of the virus, HBeAg does not appear, at first sight, to play a role in the infection. However, it may be involved in the maintenance of a state of tolerance to the virus (as in chronic carriers) and in evasion of the immune response [Carman et al., 1993]. HBeAg and HBcAg share a number of amino acids [Carman and Thomas, 1993; Salfeld et al., 1989] and are highly cross-reactive at the T-cell level [Milich et al., 1987], although they have different antigenic properties with minor serological cross-reactivity [Salfeld et al., 1989]. It may be possible, however, that the immune response mounted against one might affect the other. It was with this in mind that this study was undertaken to deal with the avidity of antibodies produced by virtually all serological categories of HBV-infected patients (anti-HBc antibodies) and to relate the maturation of these antibodies to the important "e" status of the patient.

In the cases of acute, resolving HBV infection studied here, where the date of onset of symptoms was known, the maturation of the immune response, in terms of an

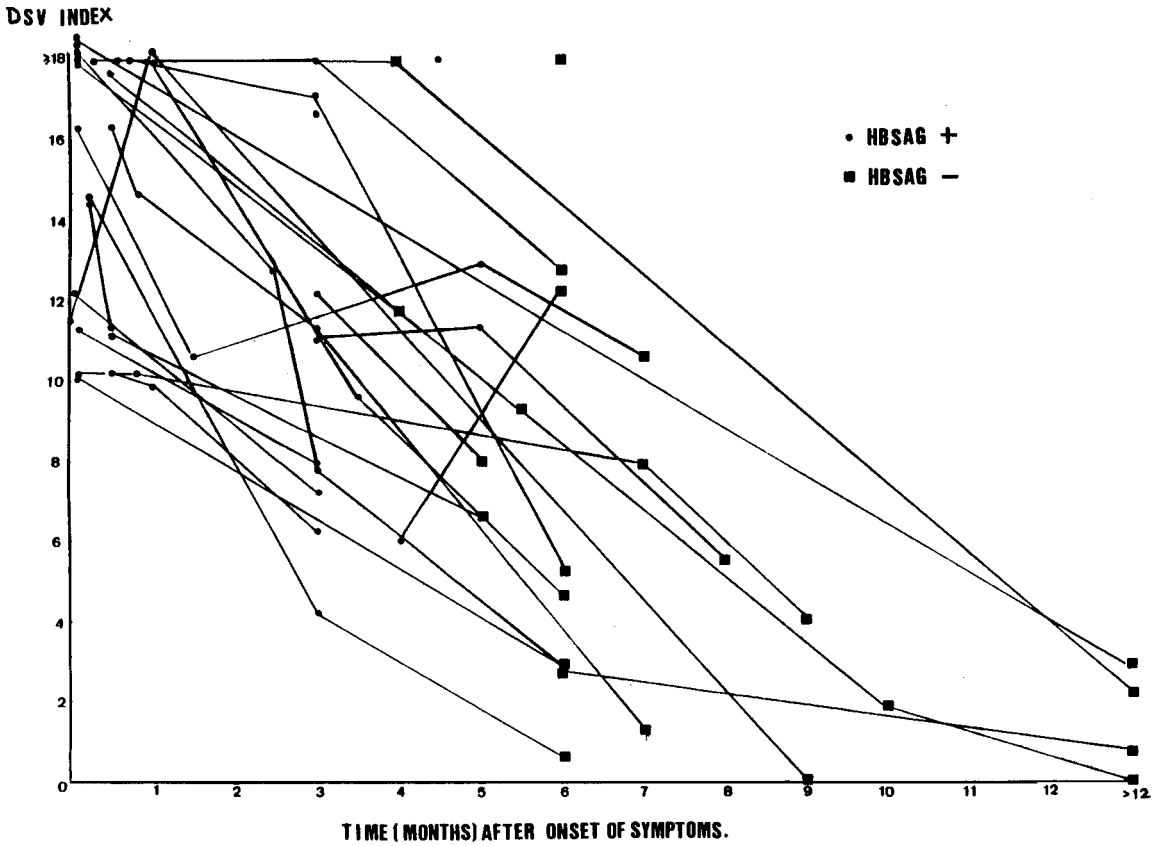

Fig. 1. Avidity (DSV index) of anti-HBc IgG in sera from cases of acute, resolving HBV infection in relation to time (in months) after onset of symptoms. ● HBsAg-positive sera; ■, HBsAg-negative sera. Lines join results obtained from sequential sera from individual cases: ●—● are data from sequential sera from cases who remained HBsAg-positive during the period of study. ●—■ are data from sequential sera from cases who converted from HBsAg-positive to HBsAg-negative during the course of the study. ■—■ are data from sequential sera from cases who were already HBsAg-positive at the start of the period of study.

TABLE III. Anti-HBc IgG Avidity Results in Sera From HBsAg-positive Cases Seroconverting From HBeAg-positive to Anti-HBe Antibody-positive Status, in Relation to Their "e" Status at the Time the Sera Were Taken

	HBeAG/anti-HBe status		
Anti-HBc IgG avidity	HBeAG+ (n = 10)	anti-HBe+ (n = 24)	HBeAG/anti-HBe– (n = 8)
DSV +	1 (10)[a]	0	0
DSV +/–	0	2 (8)	0
DSV –	9 (90)	22 (92)	8 (100)
AI +	0	0	0
AI +/–	0	0	0
AI –	10 (100)	24 (100)	8 (100)

DSV, diethylamine shift value test results; AI, avidity index results. + denotes the presence of low avidity specific anti-HBc IgG; +/– denotes an equivocal result; – denotes that the avidity of the specific anti-HBc IgG was indistinguishable from that seen in sera from cases of infection in the distant past (remote cases).
n, number of sera in the group. Numbers in parentheses are percentages.
[a]Further details of this case are to be found in Table IV (case G.C.).

increase in anti-HBc antibody avidity, took place over a period of about 6 months. This is not too disimilar from the maturation rate observed in rubella and VZV infections or of anti-gp41 in HIV infection [Thomas et al., 1990, 1992, 1996]. Only three of 11 (27%) sera remained positive for low avidity anti-core antibody by the more sensitive DSV method at 6–7 months post-onset of symptoms. One gave an equivocal result at 8 months and none was either positive or equivocal for low avidity anti-HBc IgG beyond that time. All of the HBsAg-positive acute sera contained low avidity anti-HBc antibody by both methods. Of the 40 HBsAg-negative sera, 21 (52.5%) still contained low avidity antibody by the DSV method (nine by the AI method) but 14 were completely negative for low avidity anti-core antibody by the DSV method, suggesting that maturation of the anti-core antibody response is not linked with events which control the presence or absence of HBsAg.

Comparison between HBeAg status and anti-HBc antibody avidity in acute cases showed that low avidity anti-HBc IgG was present in sera containing HBeAg or anti-HBe IgG (in 97.6% of the former and in 78.9% of the latter group). However, high avidity anti-HBc IgG was more likely to be found in anti-HBe-positive sera than in HBeAg-positive sera. The explanation for this last observation is probably a simple one: the anti-HBe-positive sera are from cases in the later stages of acute, resolving infection. However, the high incidence of low

TABLE IV. A Selection of Cases Where an "e" Antigen-positive Serum Was Available for Avidity Studies and Where the Seroconversion From "e" Antigen-Positive to "e" Antibody-Positive Status Could Be Related to Anti-Core Antibody Avidity

Patient	"e" ag	"e" ab	Anti-HBc IgM	Anti-HBc IgG avidity (DSV)	Time[a] (months)
G.H.	+	–	+/–	High	
	–	–	+/–	High	
	–	+	–	High (5 months)	5
P.	+	–	–	High	
+	–	–		High	
	–	–	–	High	
	–	–	–	High	
	–	+	–	High (37 months)	26
G.R.	+	–	–	High	
	–	+	–	High (2 months)	2
N.W.	+	–	–	High	
	–	+	–	High (11 months)	11
A.L.	+	–	N.K.	High	
	–	+	N.K.	High (81 months)	81[b]
C.	+/–	–	N.K.	High	
	Very low	–	N.K.	High (12 months)	12
G.C	+	–	N.K.	Low (AI high)	
	–	+	N.K.	High	18
D.J.	+	–	N.K.	Serum not available	
	–	–	N.K.	High	
	–	–	N.K.	High	
	–	–	N.K.	High	
	–	+	N.K.	High (23 months)	23

[a]Time (in months) between the last serum known to be HBeAg positive and the first serum known to be anti-HBe IgG positive.
[b]No sera taken between these two.
Months in parentheses indicate the length of time that high avidity anti-HBc had been detectable in sera from the patient. N.K., not known.

avidity anti-HBc IgG antibodies in sera containing either HBeAg or anti-HBe IgG suggests that in acute, resolving cases of HBV infection there is no correlation between HBeAg status and anti-HBc IgG avidity. This is in marked contrast to the evidence seen in chronic HBV carriers.

A maturation of the anti-HBc IgG response in non-resolving carrier patients was not always seen. Among the HBeAg-positive sera, just over half (50.7%) were shown to contain low avidity anti-core IgG by the DSV method (plus another 19.8% which gave equivocal results). In contrast, neither method used in this study was able to detect low avidity anti-HBc IgG in sera from carriers who were anti-HBe-positive (cf., low avidity anti-HBc IgG antibodies were detected in 78.9% of acute sera containing anti-HBe antibodies by the DSV method). Only two sera from this carrier group gave equivocal results by the more sensitive DSV method. These results suggest, therefore, that there might be a link between the appearance of high avidity anti-HBc antibodies and the disappearance of HBeAg or the appearance of anti-HBe antibodies in carriers. Although HBcAg has been shown to function both as a T cell-dependent as well as a T cell-independent antigen, whilst HBeAg is T cell-dependent only [Milich and McLachlan 1986; Milich et al., 1988], cross-reactions between the two antigens have been observed at the T-cell level [Milich et al., 1987]. Thus, an event which may expand T-cell populations reactive for HBcAg might also expand T-cell populations reactive for HBeAg and so contribute to the removal of HBeAg and the appearance of anti-HBe in the carrier group.

The correlation between high avidity anti-HBc IgG and HBeAg seroconversion is interesting. In cases which seroconverted from HBeAg-positive to anti-HBe-positive, the avidity of the anti-HBc antibody was high before the appearance of anti-HBe and before the disappearance of the "e" antigen, often by several years. About 50% of chronic patients (HBeAg-positive) had high avidity anti-HBc IgG. It is tempting to suggest that seroconversion would occur at some time in the near future in these cases also. Further follow-up is needed for confirmation. However, it seems likely that the appearance of high avidity anti-HBc in these seroconverting patients might parallel a Th-cell response to HBcAg or HBeAg and reflect an increase in the quantity and/or quality of T-cell help, being thus indicative of a more "effective" immune response which will result in the appearance of anti-HBe.

The results of this study show three things. Firstly, those who recover from the infection lose their HBsAg and go on to produce anti-HBs, showing an avidity maturation in their anti-core antibody response similar to that seen in other viral infections studied (rubella, VZV, HIV gp41), suggesting that their T-cell response to the viral protein core antigen is intact and functional. This maturation appears to be independent of both HBsAg and HBeAg status. Secondly, whilst approximately 50% of those who remain carriers and are HBeAg-positive did not show a maturing anti-HBc response over the time period of the observations (at least in so far as avidity is concerned), all anti-HBe-positive chronic cases and approximately 50% of HBeAg-positive cases had high avidity anti-HBc IgG, indicative of a mature response. Thirdly, it would appear that a seroconversion from HBeAg-positive to anti-HBe an-

tibody-positive is preceded by the appearance of high avidity anti-HBc IgG antibodies. It is tempting to suggest that the two events are related.

Whilst the appearance of anti-HBe antibodies in carriers could be due to a lowering of HBeAg levels by cross-reacting anti-HBc antibodies of higher avidity than were previously present, resulting in the removal of the tolerising potential of high levels of antigen, the appearance of anti-HBe seems to coincide with the selection of mutant viruses which can no longer produce HBeAg [Carman et al., 1993]. However, the results contained in this paper suggest that the appearance of high avidity anti-HBc antibodies may play an important role in the events which lead up to the HBeAg-to-anti-HBe seroconversion. Further studies are needed on cases where the timing of the emergence of mutants, the viral load, and other parameters would be of value. Studies on the relationship between antibody avidity, viral load, treatment, and pathology are in hand.

ACKNOWLEDGMENTS

The author is indebted to Dr E. Gould and his colleagues at the Institute of Virology, Oxford, UK, for the very generous gift of hepatitis B core antigen used throughout this study.

REFERENCES

Austyn JM (1989): Antigen-presenting cells. In Male D (ed): "Focus Series." IRL Press, Oxford.

Berek C, Ziegner M (1993): The maturation of the immune response. Immunology Today 14:400–404.

Blum HE (1993): Hepatitis B virus: Significance of naturally occurring mutants. Intervirology 35:40–50.

Brown SE, Howard CR, Zuckerman AJ, Steward MW (1984): Determination of the affinity of antibodies to hepatitis B surface antigen in human sera. Journal of Immunological Methods 72:41–48.

Careoda F, de Franchis R, Monforte AD'A, Vecchi M, Rossi E, Primignani M, Palla M, Dioguardi N (1982): Persistence of circulating HBsAg/IgM complexes in acute viral hepatitis, type B: An early marker of chronic evolution. Lancet ii:358–360.

Carman WF, Thomas HC (1993): Implications of genetic variation on the pathogenesis of hepatitis B virus infection. Archives of Virology 8 (suppl):143–154.

Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC (1989): Mutation preventing formation of e antigen in patients with chronic HBV infection. Lancet ii:588–591.

Carman W, Thomas H, Domingo E (1993): Viral genetic variation: Hepatitis B virus as a clinical example. Lancet 341:349–353.

Devey ME, Steward MW (1980): The indution of chronic antigen-antibody complex disease in selectively bred mice producing either high or low affinity antibody to protein antigens. Immunology 41: 303–311.

Devey ME, Bleasedale K, Lee S, Rath S (1988): Determination of the functional affinity of IgG1 and IgG4 antibodies to tetanus toxoid by isotype-specific solid-phase assays. Journal of Immunological Methods 106:119–125.

Eddleston ALWF (1993): Virus- and immune-mediated liver damage in hepatitis. Intervirology 35:122–132.

Eisen HN, Siskind GW (1964): Variations in affinities of antibodies during the immune response. Biochemistry 7:996–1008.

Ferrari C, Penna A, Sansoni P, Giuberti T, Neri TM, Chisari FV, Fiaccadori F (1986): Selective sensitisation of peripheral blood lymphocytes to hepatitis B core antigen in patients with chronic active hepatitis type B. Clinical and Experimental Immunology 67:497–506.

Foote J, Milstein C (1991): Kinetic maturation of an immune response. Nature 352:530–532.

Gershon RK, Paul WE (1971): Effect of thymus-derived lymphocytes on amount and affinity of anti-hapten antibody. Journal of Immunology 106:872–874.

Hedman K, Rousseau SA (1989): Measurement of avidity of specific IgG for verification of recent primary rubella. Journal of Medical Virology 27:288–292.

Hoofnagle JH, Gerety RJ, Barker LF (1973): Antibody to hepatitis B virus core in man. Lancet ii:869–873.

Inouye S, Hasgawa A, Matsuno S, Katow S (1984): Changes in antibody avidity after virus infections; detection by an immunosorbent assay in which a mild protein-denaturing agent is employed. Journal of Clinical Microbiology 20:525–529.

Kangro HO, Manzoor S, Harper DR (1991): Antibody avidity following varicella-zoster virus infections. Journal of Medical Virology 33: 100–105.

Liu Y-J, Johnson GD, Gordon J, MacLennan ICM (1992): Germinal centres in T-cell dependent antibody responses. Immunology Today 13:17–21.

Llunggren K, Hansson BG, Nordenfelt E (1991): HBsAg/IgM complexes as a prognostic marker of chronicity in acute hepatitis B infection. Scandinavian Journal of Infectious Diseases 23:529–534.

Maruyama T, McLachlan A, Iino S, Koike K, Kurokawa K, Milich DR (1993): The serology of chronic hepatitis B revisited. Journal of Clinical Investigation 91:2586–2595.

McRae B, Lange JAM, Ascher MS, de Wolf F, Sheppard HW, Goudsmit J, Allain J-P (1991): Immune response to HIV p24 core protein during the early phases of human immunodeficiency virus infection. AIDS Research and Human Retroviruses 7:637–643.

Milich DR, McLachlan A (1986): The nucleocapsid of hepatitis B virus is both a T-cell independent and a T-cell dependent antigen. Science 234:1398–1401.

Milich DR, McLachlan A, Moriarty A, Thornton GB (1987): Immune response to hepatitis B virus core antigen (HBcAg): Localisation of T cell recognition sites with HBcAg/HBeAg. Journal of Immunology 139:1223–1231.

Milich DR, McLachlan A, Stahl S, Wingfield P, Thornton GB, Hughes JL, Jones JE (1988): Comparative immunogenicity of hepatitis B virus core and e antigens. Journal of Immunology 141:3617–3624.

Nossal GJV (1993): Life, death and the immune system. Scientific American 269:52–62.

Radkowski M, Laskus T, Goch A, Slusarczyk J (1993): Affinity of anti-gp41 antibody in patients infected with human immunodeficiency virus type 1. European Journal of Clinical Investigation 23:455–458.

Rizzo LV, DeKruff RH, Umetso DT (1992): Generation of B cell memory and affinity maturation. Induction with Th1 and Th2 clones. Journal of Immunology 148:3733–3739.

Salfeld J, Pfaff E, Noah M, Schaller H (1989): Antigenic determinants and functional domains in core antigen and e antigen from hepatitis B virus. Journal of Virology 63:798–808.

Steward MW (1979): Chronic immune complex disease in mice: The role of antibody affinity. Clinical and Experimental Immunology 38:414–423.

Steward MW (1981): The biological significance of antibody affinity. Immunology Today 2:134–140.

Steward MW, Steensgard J (1983): "Antibody Affinity: Thermodynamic Aspects and Biological Significance." Boca Raton, FL: CRC Press.

Steward MW, Stanley CM, Dimarchi R, Mulcahy G, Doel TR (1991): High-affinity antibody induced by immunisation with a synthetic peptide is associated with protection of cattle against foot-and-mouth disease. Immunology 72:99–193.

Thomas HIJ, Morgan-Capner P (1988): Rubella-specific IgG subclass avidity ELISA and its role in the differentiation between primary rubella and rubella reinfection. Epidemiology and Infection 101: 591–598.

Thomas HIJ, Morgan-Capner P (1991): Rubella-specific IgG1 avidity: A comparison of methods. Journal of Virological Methods 31:219–228.

Thomas HIJ, Morgan-Capner P, Meurisse EV (1990): Studies on the avidity of IgG1 subclass antibody specific for varicella-zoster virus. Serodiagnosis and Immunotherapy in Infectious Disease 4:371–377.

Thomas HIJ, Morgan-Capner P, Enders G, O'Shea S, Caldicott D, Best JM (1992): Persistence of specific IgM and low avidity specific

IgG1 following primary rubella. Journal of Virological Methods 39:149–155.

Thomas HIJ, Morgan-Capner P, Cradock-Watson JE, Enders G, Best JM, O'Shea S (1993): Slow maturation of IgG1 avidity and persistence of specific IgM in congenital rubella: Implications for diagnosis and immunopathology. Journal of Medical Virology 41:196–200.

Thomas HIJ, Wilson S, O'Toole CM, Lister CM, Saeed AM, Watkins RPF, Morgan-Capner P (1996): Differential maturation of avidity of IgG antibodies to gp41, p24 and p17 following infection with HIV-1. Clinical and Experimental Immunology 103:185–191.

Webster RG (1968): The immune response to influenza virus: Changes in the avidity and specificity of early IgM and IgG antibodies. Immunology 14:39–52.

Wen YM, Duan SC, Howard CR, Frew AF, Steward MW (1990): The affinity of anti-HBc antibodies in acute and chronic hepatitis B infection. Clinical and Experimental Immunology 79:83–86.